IVD CE

ELECTROPHORESIS OF LIPOPROTEINS
on agarose gels

Principal and Methodology

The determination of serum lipoproteins has been utilized in studies on the genesis of atherosclerosis, response to hormonal levels, congenital and developing anomalies and genotypes. Lipoproteins differ in molecular size and because of their negative change, at pH 8.6, they migrate, in an electrical field, toward the anode.

Agarose gel electrophoresis, in comparison to other techniques, requires less time for completion and is very useful because the migration rate of the lipoproteins is much more reproducible from run to run.

HELLABIO Agarose Gels enable the separation of lipoproteins in chylomicron, β-, pre-β-, (a) and α-lipoproteins.

Required Reagents and Equipment's included in each kit:
[Warning: All reagents from each kit must be used together]

Product	KIT LE10 /100 TESTS	KIT MLE / 48 TESTS
Agarose Gels	10	16
Electrophoresis Buffer [50X concentrated]	20 ml	20 ml
Staining solution [stock solution]	2 ml	2 ml
Gel Blotter Strips	20	32
Sample Templates	10	16
Instructions for use in English		

All reagents must be used according to the instructions and until the expiration date indicated on the kit.

Preparation of reagents, storage and stability included in the kit:

a) Agarose gels: Agarose Gels are in non- barbital buffer and other non-reactive ingredients for long stability and optimum resolution of protein fractions. The Gels must be stored at **15 - 25 ^{0}C on horizontal position** until the expiration date indicated on the kit. Do not freeze the gels. Carefully discover the gel just before use and follow the instructions of the manual.

b) Electrophoresis Buffer: Non-barbital buffer and other non-reactive ingredients. It is in concentrated solution. It must be stored at 15 – 25 ^{0}C until the expiration date indicated on the kit. **To prepare working solution dilute the content of the bottle with deionized water to a final volume of 1 litre.** The diluted solution is enough for electrophoresis of all gels of the kit. The buffer solution is for one use only.

Store the diluted solution at room temperature until the expiration of the kit. If crystals appear, place the vial in warm water to dissolve the crystals.

c) Sudan black B stock solution:

Working solution			
	Propanol	**Stock solution**	**Dest.H_2O**
LE10	**5 ml**	**160 µl**	**6 ml**
MLE	**2.5 ml**	**80 µl**	**3 ml**

Alternatively use **ethanol instead of propanol** as follow:

Working solution			
	Ethanol	**Stock solution**	**Dest.H_2O**
LE10	**6 ml**	**75 µl**	**5 ml**
MLE	**3ml**	**40 µl**	**2,5ml**

Add the propanol/ethanol and the stock solution in a vial and mix it thoroughly. Then add the H_2O.Mix it very well and let is sit for at least 30 minutes. The solution is stable for 12 hours in room temperature.

d) Gel Blotter strips: Thin filter paper strips to blot the gel in the application area. Blot just for 5 seconds. Avoid humidity.

e) Destaining solution: 40% v/v propanol or 60% v/v ethanol (not included in the kit).

Additional reagents and equipments which can be provided by Hellabio:

Power supply, Electrophoresis tank, Staining-destaining baths, HellabioScan (Gel Analyzer).

Collection and Handling of Specimens

Collect 12 hours fasting blood specimens in a vacutainer tube containing 1.5 mg Na_2 EDTA per ml blood. ***Do not use heparin*** *because of its adverse effect on the lipoproteins pattern. Serum can be used too.*

The Lipoproteins are generally instable. To protect them the serum or plasma should be separated from the red blood cells within 2 hours after collection.

The β- and α-lipoproteins remain relatively constant over at lest 28 days at 4 ℃. However, there is a decrease in the mobility of the pre-β-lipoproteins. The change is most rapid in the first five days.

Freezing of the plasma irreversibly alters the lipoprotein pattern and therefore it must be stored at 4 ℃. Ideally electrophoresis of LP should he performed on the day of collection.

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Procedure of Lipoprotein Electrophoresis

a) Before the electrophoresis process prepare the staining and destaining solution according to the preparation instructions. Use undiluted serum or plasma.
b) Fill the electrophoresis chamber with adequate volume (it depends on the chamber volume) of electrophoresis buffer.
c) Take the agarose gel out of its packaging, uncover it from the plastic plate and put it on the backside of the plate in horizontal position. Keep the plastic tray **(for staining)**.
d) Blot the gel for 5'' with a gel blotter strip on the sample application zone.
e) Place the sample template on the application zone. Rub the template with forefinger so that it gets contact with the gel surface.
f) Using a 5 µl pipette, apply 5 µl of the samples across each corresponding slit and let them absorb into the gel for **5 minutes**. The application of the samples should be done as quickly as possible. The application slits should not be allowed to dry.
g) Blot the excess sample with a gel blotter strip, gently remove both the sample template and the gel blotter strip and discard them.
h) Place the gel into the tank in the right charge position, connect the tank to the power supply and run for ***20 minutes/ 100 Volts***.
i) Following the electrophoresis, switch off the power supply, put the gel on the plastic tray and dry it with hot air (less than 85 °C).
j) Place the plastic tray with the gel on a horizontal position and stain it according to the preparation instructions. Fill the plastic tray with the corresponding staining solution, and stain the gel for ***4 minutes***.
k) After the staining procedure, decolonise the gel with destaining solution ***two times for 3 minutes each***.
l) Dry again the gel and interpret the results visually or by HellabioScan or a densitometer (520 nm).

Expected values:

The values presented are those for serum lipoprotein electrophoresis on the **Hellabio agarose gel**. The normal range values for Hellabio Agarose gels were determined by calculating the mean value +/-2 standard deviation for each protein fraction from a population of 200 apparently healthy male and female adult blood donors.

It is recommended for each laboratory to establish its own normal range values in its own densitometer just one time.

Interpretation of the results:

The qualitative interpretation of the results easily can be made visually. For a quantitative interpretation of the result HellabioScan or a densitometer (520-600 nm) can be used

Limitation / Caution:

-Do not use the agarose gel film if it seems to be dried.
-Do not freeze the agarose gel film.
-Store the kit in horizontal position.
-The serum albumin is located anodic to α-Lp and can be stained lightly with staining solution. By analysis this band should not be included in the α-Lp band

Calibration:

The estimation of the electropherogram for Lipidemia can be easily done visually. For a quantitative analysis densitometer (520 - 600nm) must be used.

Exemplary serum lipoprotein separation

Fraction of lipoproteins	Expected migration position of lipoproteins	Normal value
	(+)	
a-Lp		22-46
Lp(a)		--
Pre β-Lp		0-27
β-Lp		47-71
Chylomicron	**(-)**	--

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